REMARKS

In the Office Action, claims 29-31 are withdrawn from consideration as being directed to a non-elected invention (but please see *infra*).

The Office Action acknowledges that the claims examined are free of art for the elected invention.

Claim 32 is objected to for comprising non-elected subject matter.

Claims 13-15, 23-26, 32, 35-39 and 41-47 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement.

Non-elected claims 29 and 31 are herein cancelled without prejudice. Claims 30 and 32 are herein amended to eliminate the dependency from non-elected claim 29.

Claims 13-15, 35-39 and 41-47 are herein cancelled without prejudice to accelerate the prosecution of the case.

Claims 23-26, 30 and 32 are pending in the case.

Reconsideration of present application in view of the foregoing amendments and the remarks below is respectfully requested.

Withdrawal of Non-Elected Claims

The Office Action states that claims 29-31 are withdrawn from consideration as being directed to a non-elected invention.

Applicants respectfully submit that claim 30 is not entirely directed to a non-elected subject matter to the extent that it also depends from claim 23.

Claim 30 is herein amended to eliminate the dependency from non-elected claim 29 so that it depends from claim 23 only and, therefore, is directed to the elected subject matter.

Accordingly, please consider the merit of claim 30 in the present examination.

Claim Objections

Claim 32 is objected to for comprising non-elected subject matter.

Claim 32 is herein amended to eliminate the dependency from claim 29.

Accordingly, the objection of claim 32 should be withdrawn.

Claim Rejections under 35 U.S.C. § 112

Claims 13-15, 23-26, 32, 35-39 and 41-47 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement.

Claims 13-15, 35-39 and 41-47 are herein cancelled without prejudice to solely accelerate the prosecution of the case. Accordingly, the rejection of these claims are now moot. Applicants expressly reserve a right to pursue the cancelled subjects matter in a continuation application.

The Examiner considers each factor as set forth in *In re Wands*, 858 F.2d at 737. Applicants respond below for each item with regard to claims 23-26, 30 and 32.

(1) The breadth of the claims

Specifically, the Office Action states that "[t]hese claims are broad because of the range of diseases and disorders encompassed, the levels of therapy, the variety of subjects, and the range of administration routes".

Claim 23 is herein amended to recite in the relevant portion: "A method of treating bone fracture non-union or segmental bone defects, where bone regeneration is required, in an immunocompetent subject, said method comprising administering directly to a skeletal muscle, of the subject, adjacent to said fracture or defects . . . ." Support for the

amendment can be found, for example, at page 4, lines 29-31; page 6, lines 3-5; page 24, lines 21-29; and page 39, line 15 through page 40, line 13, of the present specification.

The Office Action further states that "these claims are broad for encompassing administration of nucleic acids comprising a viral vector, along with separate administration of a promoter operably linked to a coding sequence"; and that "[s]uch administrations are doubled, due to the presence of a second viral vector in claims 23-26 and 29-30."

Claim 23 is herein amended for clarification to recite in the relevant portion: ". . . a first nucleic acid molecule comprising an adeno-associated viral vector carrying a first promoter, and a second nucleic acid molecule comprising an adenoviral vector carrying a second promoter, ..." Support can be found, for example, in Figure 2 showing a schematic diagram of recombinant AAV-BMP2 vector, and at page 26, lines 14-20.

No new matter has been introduced by the amendments.

Furthermore, It is well within the skill and knowledge of the Artisan to determine the amount of each vector required for other subjects, including humans, based on the dosages disclosed in the present application for rats.

(2) The amount of direction and guidance provided by the specification

The Office Action states that the claimed subject matter is not reasonably predictable "because the transgene is not reasonably predicted to be therapeutic in any particular disorder, and for those it is applicable to, it is not reasonably predicted to be therapeutic in any particular disorder, and for those it is applicable to, it is not reasonably predicted that enough of the target cells will be transformed and express enough stable and functional mRNA and protein therefrom, for a long enough period of time to effect treatment."

Applicants respectfully disagree with the statement.

As disclosed in Sections 7.4 and 7.5 of the present specification, the experiments with the immunocompetent rats injected with AAV-BMP2 (10^{12} viral particles or VP) and those coinjected with AAV-BMP2 ($5x10^{11}$ VP) and Ad-BMP ($5x10^{8}$ VP), demonstrated the formation of new bone, not "inappropriate bone-like structure" as the Examiner asserts, around the sites of the injections. As shown by the hematoxylin and eosin staining, the newly formed bone structures were identified within the muscle tissues and characterized by a well-defined cortical rim, trabeculae structure, and bone marrow cavity containing many adipocytes (*see* Figures 9A-9F, 13A and 13 B). Thus, when the muscle in close proximity to the fracture is injected with these vectors, the chance that the new bone is formed and fused into the endogenous bone is reasonably high.

Indeed, recombinant BMP2 has long been shown to induce healing of segmental defects in a number of species including nonhuman primates (*see*, for example, Yasko *et al.*, 1992, *J Bone Joint Surg Am* 74:659-670). Recently, Yue *et al.* (2005, *Calcif Tissue Int* 77:395-403) demonstrated in both radiographic and histological assays that in six of six Adv-BMP2-transfected and mesenchymal cells (MSC)-implanted rats, the MSC-tricalcium phosphate composites had regenerated and bridged the bone defects 6 weeks after transplantation (*see* Figs 4 and 6).

In addition, Gafni *et al.* (2004, *Mol Ther* 9(4):587-595) demonstrated that rAAV-hBMP-2 vector is an effective means of induction and regulation of bone regeneration and repair in mice. Collectively, these data indicate that ectopic expression of BMP2 can promote bone formation in vivo.

Ectopic bone formation is in fact useful for healing bone lesions or repairing segmental defects. It has been previously demonstrated in a mesenchymal stem cell-mediated gene therapy model that recombinant human BMP2 can be used for bone tissue formation and repair (Moutsatsos *et al.*, 2001, *Mol Ther* 3:449-461). When human mesenchymal stem cells were infected with recombinant adenovirus vector encoding human BMP2 or were transfected with an expression vector carrying the human BMP2

gene, and transplanted into a segmental bone defect created in the radius of Balb/c mice, they were able to induce a complete bridging of the segmental defect. Moreover, histological analysis also revealed that a new continuous cortical plate of trabecular bone and bone marrow fused with the segmental defect edges (*see* Figs. 6 a and b, of Moutsatsos *et al.*, 2001).

Turgeman *et al.* (2001, *J Gene Med* 3:240-251) also showed that when human mesenchymal stem cells infected with Adv-BMP2 were transplanted into radial 2.5 mm segmental defects in nude mice, mesenchymal stem cells infected with Adv-BMP2 were able to regenerate and bridge the defect 45 days after transplantation in five (5) out of six (6) transplanted mice (*see* Fig. 4).

In the *in vivo* Sprague-Dawley rat model, many accumulated chondrocytes and cartilaginous matrix were surrounded by a significant infiltration of undifferentiated mesenchymal cells after injection of AAV-BMP2 vectors (Chen *et al.*, 2003, *Gene Ther* 10:1345-1353). Thus, it is reasonably predictable that the AAV-BMP2/Adv-BMP2 vectors, when injected into a bone fracture area, can also induce human mesenchymal cells to form new bone and repair the fracture.

Copies of the above-cited references are submitted herewith as a part of the Information Disclosure Statement.

As discussed above, the experiments disclosed in Sections 7.4 and 7.5 of the present specification, demonstrating the formation of new bone at the direct injection sites with BM2-expressing vectors, provide sufficient direction and guidance for the treatment of bone fracture non-union or segmental bone defects, as recited in claim 23 as amended. Guided by x-ray radiography, the Artisan can inject the AAV-BMP2/Adv-BMP2 vectors directly to the muscle adjacent to the target fracture or defects. And, as discussed above, there is ample literature that supports the ability of thus-formed new bone to repair bone lesions or segmental defects.

(3) The existence of working examples

The Office Action states that "given Applicant's demonstration of inappropriate bone-like structure formation, the Artisan would not reasonably predict treatment of anything."

Applicants respectfully disagree with the statement.

Applicants are not sure about the reason why the Examiner views the formation of the new bone as "inappropriate bone-like structure formation." As discussed above, the injection of the AAV-BMP2/Adv-BMP2 vectors directly into the muscle resulted in the formation of new bone, which was characterized by a well-defined cortical rim, trabeculae structure, and bone marrow cavity containing many adipocytes (*see* Figures 9A-9F, 13A and 13 B). In addition, the references cited above support that an ectopic bone formation is in fact useful for healing bone lesions or repairing segmental defects.

Thus, the experiments described in Sections 7.4 and 7.5 of the present specification indeed serve as working examples for the claimed invention.

(4) The nature of the invention

The Examiner points out various complications and problems with regard to gene therapy in general, including lack of persistent expression, difficulty in targeting of any specific tissue, undesirable immune responses against the vector as well as the expressed transgene, and the like.

However, the present inventors demonstrated that the direct injection of the cocktail of AAV-BMP2/Adv-BMP2 vectors resulted in the persistent and efficient expression of new bone at the exact sites where the vectors were injected. In other words, the vectors successfully infected the target tissue of interest and expressed the protein of interest without inducing any immunological responses against either the vectors or the expressed protein (*see* page 40, lines 9-13, of the present specification). This was despite

the fact that the AAV-vector used was of the serotype 2, which, the Examiner cautions, "may not transfect the tissue of interest." Furthermore, all the animals used in the experiments survived well until the conclusion of the experiments without any apparent complications, such as those often expected in gene therapy by wide dissemination and transduction of non-target tissues by vectors.

Thus, the present application has overcome many obstacles encountered by gene therapy in general.

(5) The state of the prior art

The Office Action states that "[t]he majority of the prior art for gene therapy involving BMPs involves the regeneration of bone, and there exists no art of record that such BMPs can be used to treat any disorder or disease."

Applicants respectfully disagree with the statement.

As supported by the references discussed above, an ectopic bone formation using recombinant BMP2 has been reported to be useful in repairing bone lesions or segmental defects. The obstacles, however, have been the difficulties in delivering vectors carrying BMP2 to the target cells efficiently and transforming sufficient number of target cells so that BMP2 can be stably expressed to form new bone and effectuate the repair of the defects, without triggering adverse immunological reactions in a subject.

The method of the present invention has overcome such difficulties by combining AAV-BMP2 and AdV-BMP2 vectors each carrying BMP2 gene and directly injecting them into a skeletal muscle adjacent to the defects. This resulted in stable expression of new bone at the site of the injection, indicating that the expression of the protein can be accurately targeted to the area where new bone generation is required. In addition, neither the vectors nor the expressed product caused any adverse immunological reactions in the treated animals. Thus, the present invention provides an effective solution to the obstacles encountered by the prior art.

(6) The amount of experimentation required to practice the invention

The Office Action states that "[t]he Artisan would have to perform experimentation to determine the disorders, routes of treatment, types of BMP, and whether cocktails of differing AAVs are required to effect any particular treatment, in any particular animal."

Applicants respectfully disagree with the statement. Applicants believe that no undue experimentation is necessary to practice the claimed invention as the following information is provided in the present application:

1. Disorders to be treated are bone fracture or any bone disorders where bone regeneration is needed;

2. The types of BMP to be used is BMP2;

3. The vectors to be used are the cocktail vectors of a therapeutic dose of AAV-BMP2 ($5x10^{11}$ VP) and a non-cytotoxic dose of Adv-BMP2 ($5x10^{8}$ VP). These dosages are disclosed for immunocompetent rats. In other immunocompetent subjects, such as humans, the amount of the cocktail vectors can be scaled up proportionally; and

4. No toxic or immunogenic response was detected in immunocompetent rats treated with AAV-BMP2 ($5x10^{11}$ VP) and Adv-BMP ($5x10^{8}$ VP).

Thus, in conclusion, Applicants believe claims 23-26, 30 and 32, as amended, are enabled by the present specification in reference to each factor set forth in *In re Wands* and, therefore, comply with the requirements under 35 U.S.C. § 112, first paragraph.

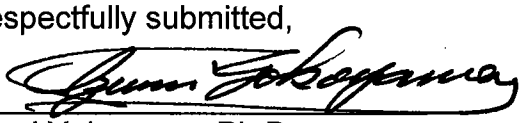
Accordingly, Applicants respectfully request that the rejection of claims 23-26, 30 and 32 under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement, be withdrawn.

Applicants believe all the pending claims are now in condition for allowance, an early notification of which is earnestly requested.

No fee is believed to be due for this submission. Should there be any deficiency in fees, please charge such fee(s) to Deposit Account No. 50-2215.

Dated: July 2, 2007

Respectfully submitted,

By 

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